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# Synopsis

Of

## D.Sc. Research work

Entitled

# Studies on Some Aspects of Morphomolecular Taxonomy and Bioinformatic Tools based Phylogeny of Butterflies of Jharkhand

to be undertaken by

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# Title

# Studies on Some Aspects of Morphomolecular Taxonomy and Bioinformatic Tools based Phylogeny of Butterflies of Jharkhand

- **STATE OF THE ART**

Biodiversity and biotechnology are the two most important fields of research and dominate over other branches in life science. Taxonomy is the base of evaluation of biodiversity by identification, and biotechnology is for their confirmation and optimization of uses and benefit.

Presently, more concern is about ecosystem service rendered by species, and for evaluation of this role of the species, taxonomy is the base.

It is expected that there will be a loss of ecosystem services equivalent to 7% of global GDP by 2050 (14000 billion dollar) due to biodiversity loss[1]**.** For prevention of this loss, it is essential to identify species and its specific role in ecosystem to get ecosystem services.

The review of literature[2-5] corroborates the "Driver and passenger hypothesis"[6] which advocates that in any ecosystem, some species play important role than the other. Those species which play important role are called driver species and others are referred to as passenger species. The driver species needs to be identified to get optimal benefit. This aspect is now in priority for both zoologists and ecologists throughout the globe.

Butterflies, particularly, so far pollination is concerned, comes as driver species and driver species are also known as keystone species[7-9]. Unfortunately, their taxonomy is not well documented in Jharkhand in particular and India in general. Further the molecular aspect of taxonomy and bioinformatics-based phylogeny has not been stared as required. Also, taxonomic identification of butterflies of India is mostly based on morpho-taxonomy, which needs to be confirmed by molecular taxonomy.

Since this aspect is unexplored on butterflies, it is essential to go for morpho-molecular taxonomy and establish the phylogeny based on bioinformatic tools.

Butterflies will be primarily identified using standard taxonomic keys.[10, 11] DNA sequencing of the samples will be done, which will be analyzed using bioinformatic tools such as BLASTN-megablast for confirmation of identification done using taxonomic keys.[12]

It is believed that butterflies (Suborder: Rhopalocera; Order: Lepidoptera), a diverse and captivating collection of insects, originated with plants and scattered around the globe in reaction to significant geological events[13] due to their high degree of complexity and diversity—they are one of the most diverse insect orders, with about 1,379 and 19,500 documented species in India and the world, respectively—they are used as model systems for ecological and evolutionary research[13, 14].

The butterflies are the second largest, most widespread, and most widely recognizable insects in phylum Arthropoda. Butterflies are significant pollinators[14] and have often been used as an indicator species for reflecting environmental changes as well as habitat conditions or structure and restoration after natural and anthropogenic disturbances, where conditions are too difficult or expensive to measure directly[15, 16].

The science of categorizing creatures is called taxonomy. With the increasing threat to biodiversity, there has never been a more pressing need for taxonomists than there is right now. A major obstacle to implementing the decisions of the Convention on Biological Diversity at both national and international levels is the scarcity of taxonomists, as stated in Decision 11/8 of the second meeting of the Conference of Parties to the Convention on Biological Diversity (CBD, 1992). The foundation of any meaningful research on biodiversity is taxonomy.[17]

Very little work has been done in Jharkhand on taxonomy and virtually no work on phylogeny of butterflies. Some workers[18-21] have attempted to study the butterflies of Jharkhand, but most of them are limited to species composition and variety, etc. but none has attempted taxonomy. Thus, no significant contribution has been made on both taxonomy as well as phylogeny till today in Jharkhand and even on national level it is in preliminary stage. No specific work has been done on molecular taxonomy of butterflies of Jharkhand. There is no report on inter-specific and intra-specific variation of genetic material of butterfly population.

Considering the above facts, this work will be undertaken as D.Sc. research work to attempt to assess the molecular taxonomy and phylogeny of butterflies of Jharkhand for the first time. It will be the first-hand novel contribution to not only the existing scientific knowledge on molecular taxonomy and phylogeny of butterflies of the state but also a significant contribution to the subject.

- **PROBLEMS TO BE INVESTIGATED**
  - Butterfly will be sampled, and the collection will be preserved in 70% of ethanol. Some dry preservation will also be done.
  - Photographs of the samples will be taken and will be geo-tagged to prepare distribution of studied samples.
  - Morpho-taxonomic study of collected samples will be done.
  - From sampled specimen DNA extraction and PCR amplification and sequencing will be done.
  - Isolated DNA will be amplified with 16s rRNA or COI (Cytochrome C oxidase subunit I) specific primer using thermal cycler.
  - BLAST search will be performed.
  - Evolutionary relationship(s) will be inferred.
  - Distance matrix will be prepared and inter and intraspecific variation will be examined
  - Phylogenetic tree will be prepared on basis of molecular studies.
  - The work will also estimate the species diversity and attempts will be made to calculate the expected number of species in a particular habitat based on Broken Stick model of niche of McArthur (1968).
  - Based on expected number species estimation, a predictive model for the population will be proposed.

- **OBJECTIVES**

The objective of the present project is to have firsthand information on morpho molecular taxonomy of the butterflies of the State with geo-tagging of each

species for mapping of distribution for the first time. The objective also includes the establishment of phylogeny of butterflies based on bioinformatic attributes which has not been done for species of the state. The research outcome will address the issues of cryptic species and phylogeny. Species diversity and apart from the actual number of species, expected number of species will be estimated.

- **HYPOTHESIS**

The study will enable to have firsthand information on the butterfly biodiversity which as per CBD (Convention on Biological Diversity)[22], is defined as ranging from gene to ecosystem. The work done will result into establishing the phylogeny of the butterfly species of Jharkhand based on bioinformatic tools. The work will throw light on evolutionary relationships between the butterfly species of Jharkhand. The research will also result into the biodiversity map of butterflies on the basis of their geo-tagging during collection from different parts of the state which is the need of the day for biodiversity conservation practices but has not been done so far. The work will also show the predictive model of butterflies' population based on expected number of species in the system when compared to actual number encountered during the study. Further the confusions pertaining to cryptic species will be addressed.

- **RESEARCH DESIGN**
  - The butterflies' samples will be collected and preserved as per previously published standard methodology[23].
  - While collecting, the samples will be photographed.
  - All photographs will be geo-tagged using appropriate software applications.
  - DNA extraction, PCR amplification and sequencing will be done.
  - Samples will be identified and classified by analyzing the sequences using various bioinformatic tools.
  - Phylogenetic tree will be prepared, and other analyses will be done.

- **METHODOLOGY**

1. Collection: butterflies will be collected using standard methods described earlier.[23]
2. Preservation of the samples will be done using standard preservation techniques[23].
3. For photography suitable camera will be used, geotagging will be done.
4. DNA extraction will be done, quality will be evaluated on Agarose Gel (1%).
5. PCR: Using a thermal cycler, extracted DNA will be amplified using a COI-specific primer.[24, 25]
6. Sequencing: Sanger sequencing will be performed after the PCR amplicon has been bead purified[24, 25].
7. For species level confirmation, BLAST (Basic Local Alignment Search Tool) will be employed with values included in scoring system of BLAST such as Max Score, query cover, E Value, Total Score, and Identity percent. BLAST results will be given as a graphic overview, alignments, and MSA, etc[12].
8. Distance matrix will be prepared using Neighbor-joining method.[12]
9. Phylogenetic tree will be prepared using the tools present in the BLAST results page.[12]
10. Accession IDs for all the nucleotide sequences will be obtained from GenBank

*** Based on above methodology preliminary work has been carried on and following accession ID has already been procured from NCBI >PP842458**

- **STATISTICAL ANALYSIS**

Data collected during sampling of population of butterflies will be statistically analyzed. Species diversity will be calculated following Shannon and Wiener[26], Hurlbert[27], Simpson[28], and evenness, following Pleiou (1975)[29]. Expected number of species in a habitat will be calculated following Llyod and Ghelardi (1964)[30], which is based on broken stick model of McArthur[31] of Niche.

- **TOOLS TO BE USED**

1. Insect net

2. Ethyl alcohol
3. Insect box
4. Ethyl acetate
5. Glass/Plastic containers
6. Centrifuge
7. Thermal cycler
8. Windows based PC and online tools
9. Software applications such as Arc-GIS, SPSS
10. Chemicals etc.

- **SIGNIFICANCE OF STUDY**

The study on butterflies of the state will a firsthand novel contribution, not only to the existing scientific knowledge on molecular taxonomy and phylogeny but also original contribution to the subject. The findings will address the issues of cryptic species and provide a clear base of phylogeny in contrast to the existing methods which are basically on the knowledge of a particular worker or a group.

- **RELEVANCE TO THE PRESENT-DAY PROBLEM AND NEED OF THE SOCIETY**

The survival as well as sustainable development of human being on the earth is dependent on biodiversity and its conservation. Taxonomy as branch of science is base of biodiversity for its study, enlistment, identification its existing position in a community and a particular habitat.

Taxonomy of a particular species is essential for priority fixation for conserving the species for economic benefit, ecosystem services, for identifying the keystone species for medicinal value, international trade and also resilience of the species under stress or in case of local extinction by fire, flood, earth quack etc.

Thus, taxonomic study, as the present one has been proposed, concerns biodiversity both on genetic and species level, social upliftment through ecosystem services by butterfly, economic development and above all human well being which underline the relevance to society.

- **CONTRIBUTION TO EXISTING KNOWLEDGE**

The inter and intra specific variation will be known which in fact form base of genetic diversity. No such works has been taken in Jharkhand. The present work will provide the firsthand information on phylogeny and molecular taxonomy of Butterflies of Jharkhand.

- **FUTURE WORK**

The outcome of the work will throw light on the distribution, phylogeny, and molecular taxonomy of butterflies of Jharkhand, which will be helpful in conservation related studies and policies of the state and country for butterflies.

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